

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a first nucleotide sequence selected from the group consisting of:
 - 5 (a) a first nucleotide sequence encoding a polypeptide of at least 25 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:18;
 - (b) a second nucleotide sequence encoding a polypeptide of at least 25 amino acids that has at least 85% identity based on the Clustal method of alignment
10 when compared to a polypeptide of SEQ ID NO:10;
 - (c) a third nucleotide sequence encoding a polypeptide of at least 40 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14;
 - (d) a fourth nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment
15 when compared to a polypeptide of SEQ ID NO:28;
 - (e) a fifth nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:24;
 - 20 (f) a sixth nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2;
 - (g) a seventh nucleotide sequence encoding a polypeptide of at least 80 amino acids that has at least 95% identity based on the Clustal method of alignment
25 when compared to a polypeptide of SEQ ID NO:6;
 - (h) an eighth nucleotide sequence encoding a polypeptide of at least 240 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:22;
 - (i) a ninth nucleotide sequence encoding a polypeptide of at least 250 amino acids that has at least 80% identity based on the Clustal method of alignment
30 when compared to a polypeptide of SEQ ID NO:16;
 - (j) a tenth nucleotide sequence encoding a polypeptide of at least 300 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:4 and 26;
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 - (k) an eleventh nucleotide sequence encoding a polypeptide of at least 500 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12; and

(l) a twelfth nucleotide sequence comprising a complement of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), or (k).

5 2. The isolated polynucleotide of Claim 1, wherein the first nucleotide sequence comprises of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 9, 11, 13, 15, 17, 21, 23, 25, and 27.

 3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are DNA.

 4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are RNA.

10 5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.

 6. An isolated host cell comprising the chimeric gene of Claim 5.

 7. A host cell comprising an isolated polynucleotide of Claim 1.

15 8. The host cell of Claim 7 wherein the host cell is selected from the group consisting of yeast, bacteria, and plant.

 9. A virus comprising the isolated polynucleotide of Claim 1.

 10. A polypeptide selected from the group consisting of:

20 (a) a polypeptide of at least 25 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:18;

 (b) a polypeptide of at least 25 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10;

25 (c) a polypeptide of at least 40 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14;

 (d) a polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:28;

30 (e) a polypeptide of at least 50 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:24;

35 (f) a polypeptide of at least 50 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2;

 (g) a polypeptide of at least 80 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6;

- 5 (h) a polypeptide of at least 240 amino acids that has at least 90 % identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:22;
- (i) a polypeptide of at least 250 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:16;
- 10 (j) a polypeptide of at least 300 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:4 and 26; and
- (k) a polypeptide of at least 500 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12.
- 15 11. A method of selecting an isolated polynucleotide that affects the level of expression of an aminolevulinic acid biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:
- (a) constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from an isolated polynucleotide of Claim 1;
- 20 (b) introducing the isolated polynucleotide into a plant cell;
- (c) measuring the level of polypeptide in the plant cell containing the polynucleotide; and
- (d) comparing the level of polypeptide in the plant cell containing the isolated polynucleotide with the level of polypeptide in a plant cell that does not contain the isolated polynucleotide.
- 25 12. The method of Claim 11 wherein the isolated polynucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 9, 11, 13, 15, 17, 21, 23, 25, and 27.
- 30 13. A method of selecting an isolated polynucleotide that affects the level of expression of an aminolevulinic acid biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:
- (a) constructing an isolated polynucleotide of Claim 1;
- (b) introducing the isolated polynucleotide into a plant cell;
- 35 (c) measuring the level of polypeptide in the plant cell containing the polynucleotide; and
- (d) comparing the level of polypeptide in the plant cell containing the isolated polynucleotide with the level of polypeptide in a plant cell that does not contain the polynucleotide.

14. A method of obtaining a nucleic acid fragment encoding an aminolevulinic acid biosynthetic enzyme polypeptide comprising the steps of:

- (a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 9, 11, 13, 15, 17, 21, 23, 25, and 27 and a complement of such nucleotide sequences; and
- (b) amplifying a nucleic acid sequence using the oligonucleotide primer.

15. A method of obtaining a nucleic acid fragment encoding an aminolevulinic acid biosynthetic enzyme polypeptide comprising the steps of:

- (a) probing a cDNA or genomic library with an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 9, 11, 13, 15, 17, 21, 23, 25, and 27 and a complement of such nucleotide sequences;
- (b) identifying a DNA clone that hybridizes with the isolated polynucleotide;
- (c) isolating the identified DNA clone; and
- (d) sequencing a cDNA or genomic fragment that comprises the isolated DNA clone.

16. A composition comprising the isolated polynucleotide of Claim 1.

17. A composition comprising the isolated polypeptide of Claim 10.

18. An isolated polynucleotide of Claim 1 comprising a nucleotide sequence having at least one of 30 contiguous nucleotides.

19. A method for positive selection of a transformed cell comprising:

- (a) transforming a host cell with the chimeric gene of Claim 5; and
- (b) growing the transformed host cell under conditions which allow expression of a polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

20. The method of Claim 19 wherein the host cell is a plant.

21. The method of Claim 20 wherein the plant cell is a monocot.

22. The method of Claim 20 wherein the plant cell is a dicot.

23. A method of altering the level of expression of an aminolevulinic acid biosynthetic enzyme in a host cell comprising:

- (a) transforming a host cell with the chimeric gene of Claim 5; and
- (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of an aminolevulinic acid biosynthetic enzyme in the transformed host cell.

24. A method for evaluating at least one compound for its ability to inhibit the activity of an aminolevulinic acid biosynthetic enzyme, the method comprising the steps of:

- 5 (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an aminolevulinic acid biosynthetic enzyme polypeptide, operably linked to at least one suitable regulatory sequence;
- 10 (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the aminolevulinic acid biosynthetic enzyme encoded by the operably linked nucleic acid fragment in the transformed host cell;
- (c) optionally purifying the aminolevulinic acid biosynthetic enzyme polypeptide expressed by the transformed host cell;
- 15 (d) treating the aminolevulinic acid biosynthetic enzyme polypeptide with a compound to be tested; and
- (e) comparing the activity of the aminolevulinic acid biosynthetic enzyme polypeptide that has been treated with a test compound to the activity of an untreated aminolevulinic acid biosynthetic enzyme polypeptide, thereby selecting compounds with potential for inhibitory activity,
- thereby selecting compounds with potential for inhibitory activity.